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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Marc Bellotti et al.	Art Unit: **1653**
Serial No. **10/796,691**	Examiner: **Robert B. Mondesi**
Filed: **March 8, 2004**	
For: **Methods and Apparatus for Creating Particle Derivatives of HDL With Reduced Lipid Content**	

DECLARATION OF MR. HASSIBULLAH AKEEFE UNDER 37 C.F.R. §1.132

I, Hassibullah Akeefe, B.Sc., do hereby declare:

1. I am one of ordinary skill in the art in the field of lipid studies. I am currently a Scientist/Research Lab Manager at Lipid Sciences Inc., Pleasanton, California. I earned a B.Sc. degree in Biochemistry in 1994 at the University of Maryland at College Park, Maryland. My *curriculum vitae* is enclosed (Exhibit A). The list of the publications is enclosed (Exhibit B).

2. I am a named inventor of U.S. Patent Application Serial No. 10/796,691 ("the present application"), and I am familiar with the present application, an article by Clay *et al.* (1999) "Formation of apolipoprotein-specific high-density lipoprotein particles from lipid-free apolipoproteins A-I and A-II." *Biochemical Journal*, v. 337, pp. 445-451 (hereinafter "Clay"), and an article by Durbin and Jonas (1999) "Lipid-free apolipoproteins A-I and A-II promote remodeling of reconstituted high density lipoproteins and alter their reactivity with lecithin:cholesterol acyltransferase." *Journal of Lipid Research*, v. 40, pp. 2293-2303 (hereinafter "Durbin").

3. I declare that enclosed herewith as Exhibits C and D are the data obtained according to my directions or under my supervision. These data characterize particle derivatives obtained by delipidation of high density lipoprotein particles obtained from a biological fluid by delipidation using a sevoflurane:n-butanol 95:5 mixture substantially as disclosed in the present application.

4. As one of ordinary skill in the art, I declare that properties of particle derivatives recited in the claims of the present application are different from those of the reconstituted particles in Clay at least because applicants' particle derivatives and the reconstituted particles in Clay are formed from different components. In Clay, apoA-I and apoA-I/apoA-II particles are formed by incubation of lipid-free apoproteins and low density lipoproteins. Applicants' HDL particle derivatives are obtained by delipidation of the HDL lipoprotein particles naturally occurring in a biological fluid, such as plasma.

5. I declare that the particles disclosed in Clay differ from applicants' particle derivatives in their lipid composition. For example, the reconstituted particles in Clay do not contain measurable triacylglycerol or non-esterified fatty acids (see Clay, p. 449, second column). The particle derivatives obtained by applicants' method are obtained by modifying naturally occurring HDL particles and inherently contain a variety of lipids from the naturally occurring HDL particles. The modification of the naturally occurring HDL particles, according to applicants' method, results in particle derivatives that contain lower levels of at least one of phospholipids or cholesterol than the naturally occurring HDL particles. Applicants' particle derivatives also contain levels of triacylglycerol (TG) comparable to those of the naturally occurring HDL particles. Applicants' particle derivatives also contain non-esterified fatty acids. See Exhibits C and Exhibit D, Figures 1 and 2.

6. I declare that the particles disclosed in Clay differ from applicants' particle derivatives in their protein composition. The HDL particles generated in Clay were formed using lipid-free apoA-I and apoA-II, and incubating them with LDL. Accordingly, the only

protein components in the Clay apoA-I-containing HDL particles are apoA-I and, optionally, apoA-II. In contrast, applicants' delipidated HDL particles inherently retain similar composition and distribution of apolipoproteins to those found in the source HDL particles found in the biological fluids. In particular, in addition to apoA-I and apoA-II, applicants' particles comprise at least one of apoC-III, apoD or apoE. See Exhibit C.

7. I declare that the particles disclosed in Clay differ from applicants' particle derivatives in their apoA-I/apoA-II ratio. In Table 2, Clay discloses the characterization of its apoA-I containing HDL particles (see p. 450 in Clay). The particles of Clay possessing the characteristics disclosed in Table 2 are different from applicants' particle derivatives. First, according to the results of two experiments reported in Table 2, the particles of Clay have apoA-I/apoA-II stoichiometric molar ratios of 1.8 and 2.9, that is, an average of approximately 2.3. In contrast, applicants' particle derivatives inherently possess apolipoprotein composition similar to that of naturally occurring particles and have an ApoA-I/ApoA-II stoichiometric molar ratio of 3.0, similar to that of intact plasma. See Exhibit C.

8. I declare that applicants' particle derivatives are distinguished from the particles in Durbin at least due to a number of structural differences. Durbin reports the effect of lipid-free apoA-I and apoA-II on the structure and properties of reconstituted HDL particles. In Durbin, the particles are reconstituted from human plasma-derived apoA-I or apoA-II with L-a-palmitoyloleoylphosphatidylcholine (POPC) and cholesterol (see Durbin, p. 2294, second column, section "Preparation of rHDL"). The particles in Durbin are generated using POPC, lipid free ApoA-I and lipid free ApoA-II. Accordingly, the particles in Durbin contain only POPC and cholesterol as their lipid components. Thus, the particles of Durbin contain a single phospholipid – POPC. Unlike the particles in Durbin, applicants' particle derivatives contain multiple phospholipids, comprising at least phosphatidylcholine (PC), phosphatidylserine (PS) or phosphatidylethanolamine (PE). See Exhibit D, Figures 3-5.

9. I declare further that all statements made herein are of my own knowledge and are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any patent issuing on this application.

Signature

Hassibullah Akeefe
Name

February 8, 2006
Date

EXHIBIT A

Hassibullah Akeefe

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Antioch, CA 94509
(H) 925-777-0595
(C) 925-642-7962

Experience:

12/2005 – Present Scientist/Research Lab Manager Lipid Sciences Inc. Pleasanton, CA

Manage R&D laboratory activities regarding research and development activities in both the selective HDL delipidation and Viral Immunotherapy platforms. Manage scientists and research associates to further develop, optimize and characterize the effects of delipidation on plasma lipoproteins and biochemistry as well as developing new and novel methods for creating vaccines for the Viral Immunotherapy Program. Execute and manage R&D laboratory and personnel in support of the human clinical trial.

6/2002 – 12/2005 Research Associate II Lipid Sciences Inc. Pleasanton, CA

Characterization and analysis of plasma lipids and protein in development of the companies selective delipidation of Plasma lipoprotein patent pending process. Development of methods for generating antigenic viral particles for HIV, SIV and SARS. In vitro and in vivo analysis and characterization of these particles. Development and characterization of a delipidation protocol for the generation of highly antigenic particles for the treatment of cancers. Assisted in the development, design, testing and validation of the delipidation system.

10/1999 - 6/15/2002 Research Associate III Avigen Inc. Alameda, CA

Characterization, construction, production, purification and analysis of various Adeno-Associated Virus (AAV) vectors in a cell based system; generation of stably transfected cell lines; work on analyzing the feasibility of various AAV serotypes in production of clinical vectors for the treatment of disease.

9/1995 - 10/1999 Research Associate I J. David Gladstone Institutes San Francisco, CA

Screening, generation and maintenance of transgenic mouse and rabbit colonies; microdissection, fixation, sectioning and immunohistochemical staining of tissue; DNA/RNA isolation; Southern, Northern and Western blotting; ELISA; generating transgenic mouse hippocampal cDNA library; minor animal surgery; managing and organizing of the lab.

7/1997 - 10/1999 Staff Research Associate
Children's Hospital Oakland Research Institute Oakland, CA

Screening, generation and maintenance of transgenic mice; characterization and analysis of transgenic mouse plasma using FPLC, purification and generation of antibodies specific for hepatic lipase; developing screening and analysis assay's (PCR, qPCR, ELISA) for the research project; management and organization of the lab supplies.

9/1994 - 6/1996 Laboratory Technician Lifescan Inc. Milpitas, CA

Testing, analysis, manufacturing, and quality control of the company's glucose strips according to the company's cGMP and cGLP guidelines.

Education:

12/1994 University of Maryland Bachelor's Degree College Park, MD

Technical Skills:

- Eukaryotic cell culture; immortalized lines and primary lines
- Bacterial culture and transformation
- Adenovirus preparation, purification, and quantification
- Molecular cloning and recombinant DNA techniques
- Southern, Northern and Western blotting and hybridization
- Small animal handling experience
- Nucleic Acid and Protein purification
- PCR/RT-PCR/qPCR
- FACS
- Column Chromatography
- GC
- Certified Hitachi 911
- Adeno-Associated Virus preparation, purification and quantification.
- Tissue fixing/ sectioning and staining.

EXHIBIT B

Publications:

1. Moiz Kitabwalla, Francois Villinger, Aftab A. Ansari, James E.K. Hildreth, Hassibullah Akeefe, Zhaohao Liao, Ann E. Mayne, Lisa Gargano, Adam P. Conner, Jo-Ann Maltais, Gretchen Kunas, and Marc Bellotti. Enhancement of cell mediated immune responses using lipid depleted lentivirus as immunogen: A novel approach for inducing recognition of new viral epitopes. Vaccine. (23). pp4666-4677, 2005.

2. H. Dichek, W. Brecht, J. Fan, Z.S Ji, S. McCormick, H. Akeefe, L. Conzo, D. Sanan, K. Weisgraber, S. Young, J.M Taylor and R.W Mahley. Overexpression of Hepatic Lipase in Transgenic Mice Decrease Apolipoprotein B-containing and High Density Lipoproteins: Evidence that Hepatic Lipase Acts as a Ligand for Lipoprotein Uptake. Journal of Biological Chemistry, Jan 1998 23;273(4): 1896-1903

3. M. Buitini, M. Orth, S. Bellosta, H. Akeefe, R.E Pitas, T.W Corray, L. Mucke and R.W Mahley. Expression of Human Apolipoprotein E3 or E4 in Neurons of ApoE Knockout Mice: Isoform-specific Effects on Age-related Neurodegeneration In Vivo, Journal of Neuroscience, Jun 1998 15; 19(12): 4867-80

4. M. Buitini, H Akeefe, C. Lin, R.W Mahley, R.E Pitas, T.W Corray, and L. Mucke. Dominant Negative Effect of apolipoprotein E4 revealed in transgenic models of neurodegenerative disease, Journal Neuroscience 1999 JUN 15; 19(12):4867-80

5. H. L. Dichek, S. M. Johnson, H. Akeefe, G. T. Lo, E. Sage, C. E. Yap, and R. W. Mahley. **Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice** J. Lipid Res., February 1, 2001; 42(2): 201 – 210

Abstracts and Presentations:

1. Inhibition of AAV Vector Transduction by Animal Sera In VitroDorothy Huey-Louie,[1] James Allen,[1] Hassibullah Akeefe,[1] Brian Christie,[1] Shang-Zhen Zhou,[1] Richard Surosky,[1] Jennifer Wellman,[1] Alan McClelland,[1] Peter Colosi.[1]

2. A Simple, Efficient, and General Method for the Production of AAV Type 1-6 VectorsShang-Zhen Zhou,[1] Brian Christie,[1] Jennifer Wellman,[1] James Allen,[1] Hassibullah Akeefe,[1] Richard Surosky,[1] Michael Lochrie,[1] Dirk Grimm,[2] Clare Thomas,[2] Hiroyuki Nakai,[2] Mark Kay,[2] Alan McClelland,[1] Peter Colosi.[1]

3. Neutralizing Activity Against Different AAV Serotypes in Sera from Untreated Humans and in Sera from Humans Treated with an AAV2 VectorRichard Surosky,[1] Dorothy Huey-Louie,[1] James Allen,[1] Shang-Zhen Zhou,[1] Brian Christie,[1] Hassibullah Akeefe,[1] Ciaran Scallan,[1] Sharon Powell,[1] Linda Couto,[1] Katherine High,[2] Mark Kay,[3] Alan McClelland,[1] Peter Colosi.[1]

4. Benefit of Delipidated Retroviruses as Potential Autologous Therapeutic Vaccines. AIDS Vaccine 2005 International Conference. Montreal, Quebec. September 6-9, 2005. Abstract#107P.M. Kitabwalla1, H. Akeefe1, A. Ansari2, F. Villinger2,A. Conner1, J.E.K. Hildreth3, and M. Bellotti1. Lipid Sciences, Inc., Pleasanton, CA1 Emory University School of Medicine, Atlanta, GA2; Johns Hopkins School of Medicine, Baltimore, MD3

5. Delipidated Retroviruses as Potential Autologous Therapeutic Vaccines-A Pilot Experiment. Keystone Symposia on HIV Vaccine Development. Banff, Alberta. April 9-15, 2005. Abstract#237. M. Kitabwalla[3], A. Ansari[1], F. Villinger[1], H. Akeefe[3], A. Conner[3], J.E.K. Hildreth[2], and M. Bellotti[3]. Emory University School of Medicine, Atlanta, GA[1]; Johns Hopkins School of Medicine, Baltimore, MD[2], Lipid Sciences, Inc., Pleasanton, CA[3]

6. Solvent-Treated Retroviruses as Novel Vaccines-A Study in Characterizing Delipidated Retroviruses. Keystone Symposia on HIV Vaccine Development. Banff, Alberta. April 9-15, 2005. Abstract# 227. J.E.K. Hildreth[1], Z. Liao[1], H. Akeefe[2], A. Conner[2], M. Bellotti[3], A. Ansari[3], F. Villinger[3], and M. Kitabwalla[2]. Johns Hopkins School of Medicine, Baltimore, MD[1], Lipid Sciences, Inc., Pleasanton, CA[2], Emory University School of Medicine, Atlanta, GA[3].

7. A Prime-Boost Immunization Strategy Using Delipidated SIV Gives Rise to a Broader CD4+ and CD8+ T-Cell Responses in Mice than AT-2 Treated or Live Virus-A Novel Therapeutic Vaccine Approach for HIV Infection. Keystone Symposia on HIV Vaccine Development. Whistler, B.C. April 11-18, 2004. Abstract#304. Ansari[1], F. Villinger[1], J. E.K. Hildreth[2], M. Bellotti[3], J. B. Maltais[3], H. Akeefe[3], T. Perlman[3], A. Conner [3], G. Kunas[3], and M. Kitabwalla[3]. Emory University School of Medicine, Atlanta, GA[1]; Johns Hopkins School of Medicine, Baltimore, MD[2], Lipid Sciences, Inc., Pleasanton, CA[3].

EXHIBIT C

Table 1.

Comparison of High Density Lipoprotein (HDL) Particles from Heparin-Manganese Supernatant of Intact Plasma and HDL Particle Derivatives Obtained from Plasma, Delipidated Using Sevoflurane:n-Butanol in a 95:5 ratio.

Composition of HDL particles from Heparin-Manganese Supernatants of Intact Plasma and Delipidated Plasma		
	Mole Ratio	
	Intact Plasma HDL	Delipidated Plasma HDL
ApoA-I / ApoA-II	3.0	3.0
ApoD / ApoA-II	0.2	0.3
ApoE / ApoA-II	0.1	0.1
ApoC-III / ApoA-II	0.4	0.3
HDL-C / ApoA-II	55.6	21.6
PL / ApoA-II	73.0	75.5
TG / ApoA-II	15.8	12.5

EXHIBIT D

Characterization of the composition of HDL Particle Derivatives Obtained from Plasma Delipidated Using Sevoflurane:n-Butanol in a 95:5 ratio.

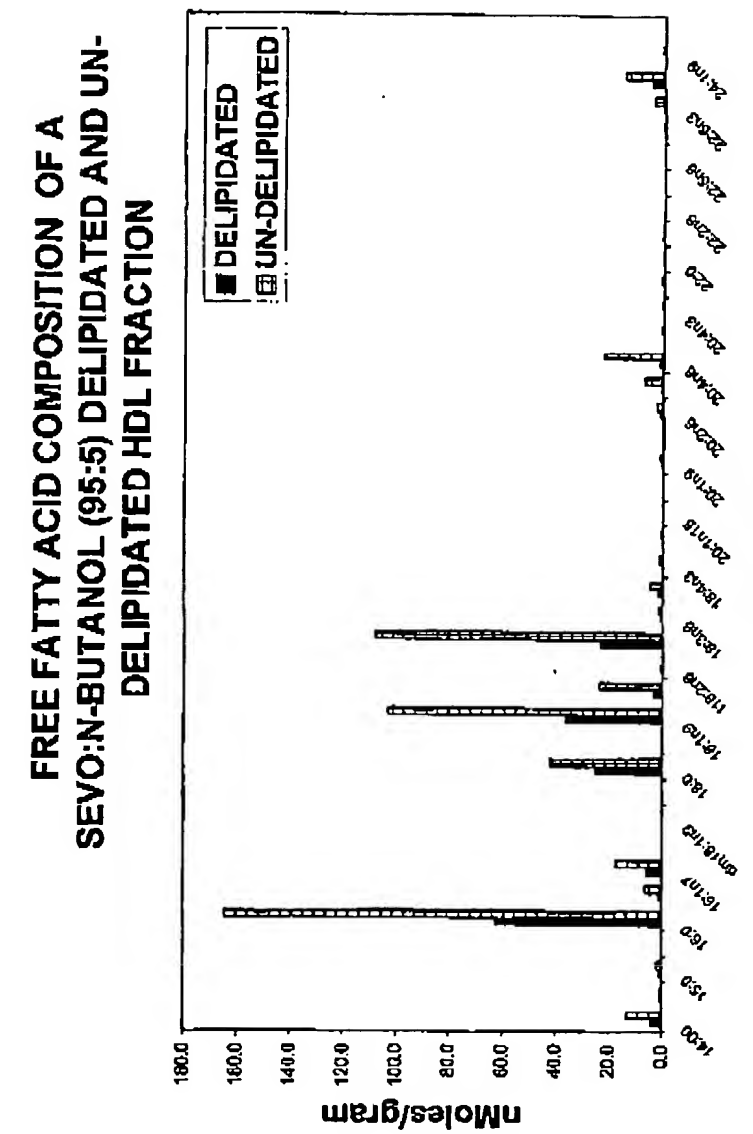

Figure 1

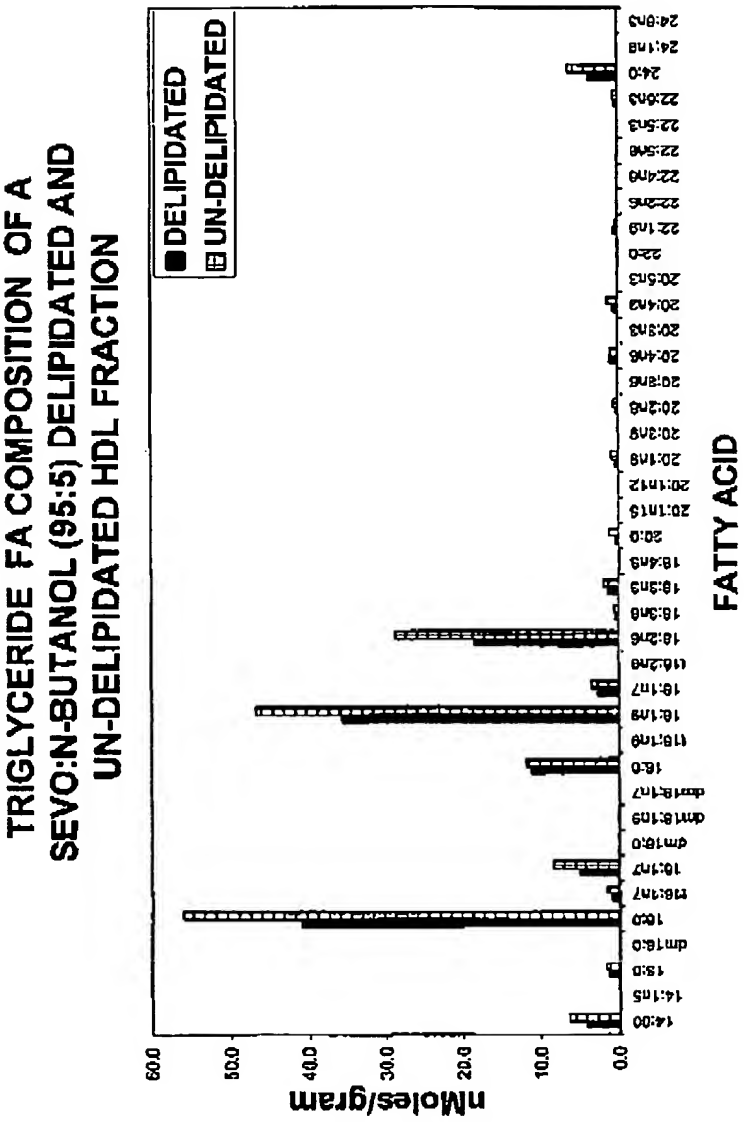
TRIGLYCERIDE FA COMPOSITION OF A SEVO:N-BUTANOL (95:5) DELIPIDATED AND UN-DELIPIDATED HDL FRACTION
DELIPIDATED
UN-DELIPIDATED
nMoles/gram
60.0
50.0
40.0
30.0
20.0
10.0
0.0
FATTY ACID

Figure 2

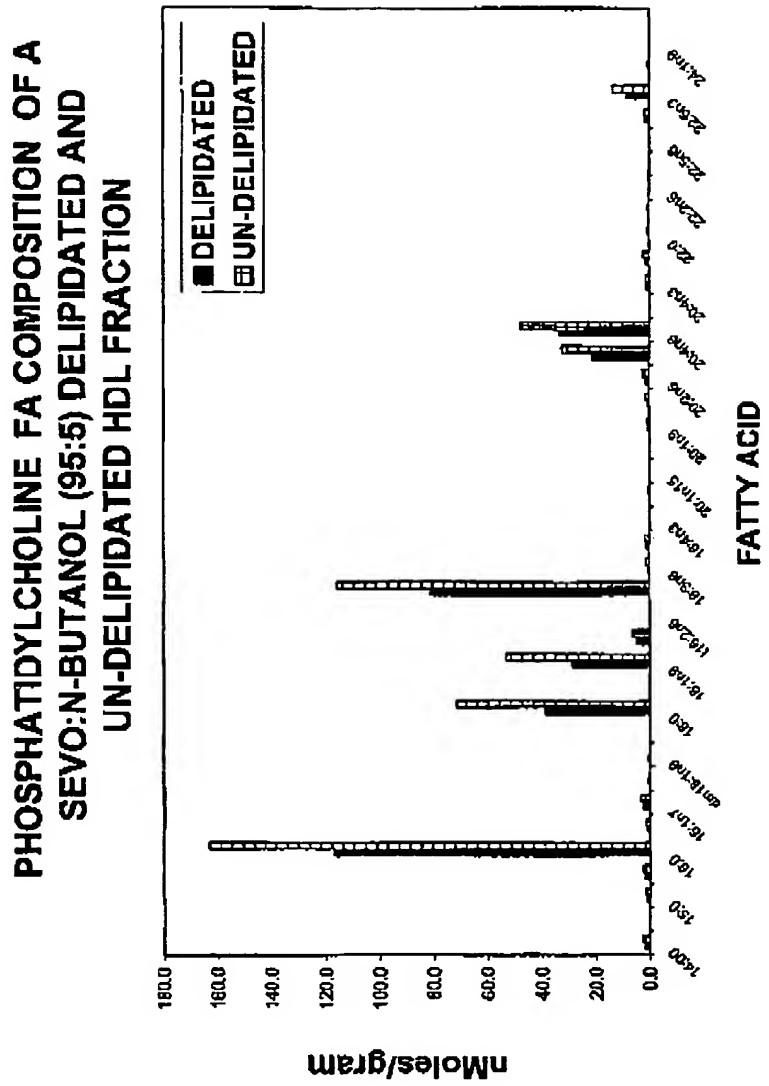
PHOSPHATIDYLCHOLINE FA COMPOSITION OF A SEVO:N-BUTANOL (95:5) DELIPIDATED AND UN-DELIPIDATED HDL FRACTION
DELIPIDATED
UN-DELIPIDATED
nMoles/gram
FATTY ACID

Figure 3

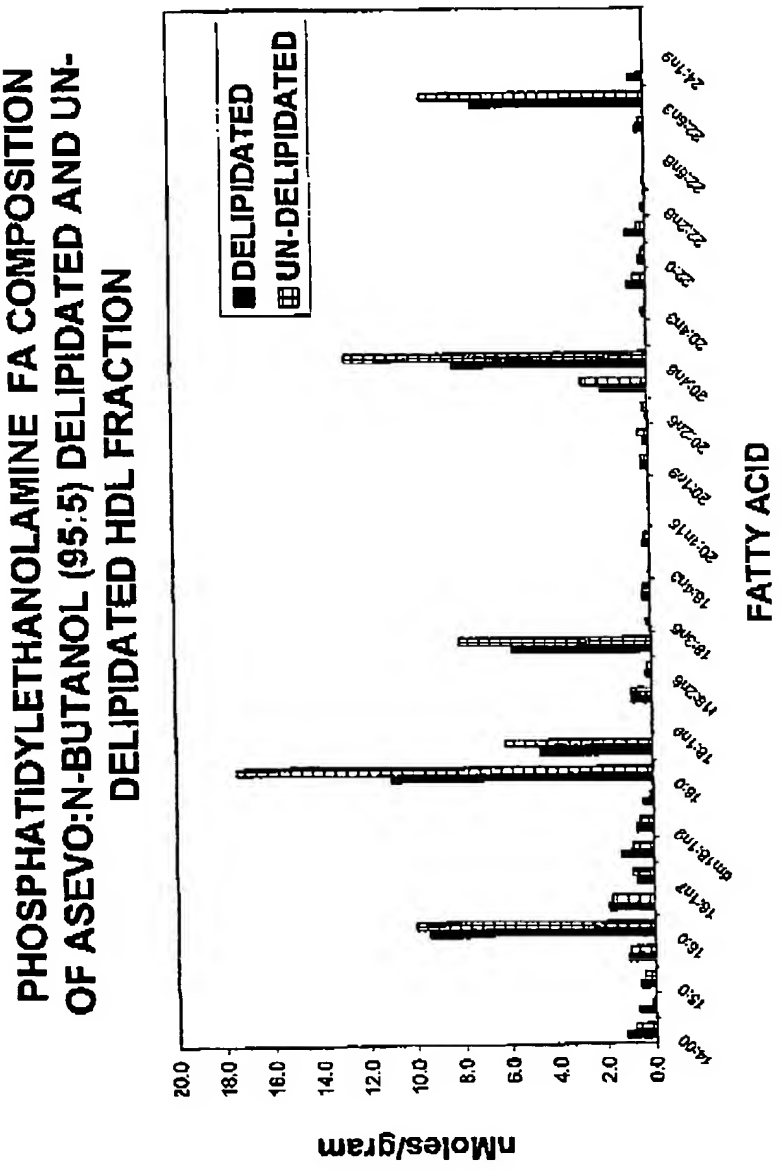
PHOSPHATIDYLETHANOLAMINE FA COMPOSITION
OF ASEVO:N-BUTANOL (95:5) DELIPIDATED AND UN-
DELIPIDATED HDL FRACTION
DELIPIDATED
UN-DELIPIDATED
nMoles/gram
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
14:00
15:0
16:0
16:1n7
6m16:1n9
16:0
16:1n9
16:2n6
18:3n6
18:4n3
20:1n15
20:1n9
20:2n6
20:4n6
20:4n3
22:0
22:2n6
22:5n6
22:5n3
24:1n9
FATTY ACID

Figure 4

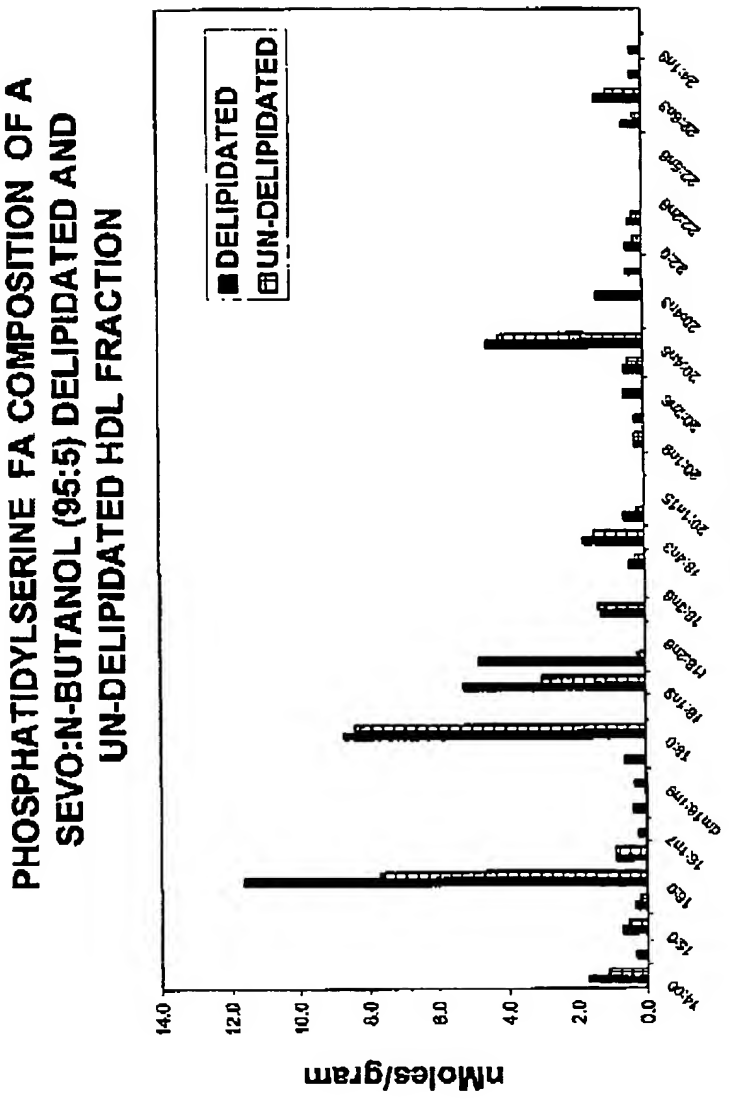
PHOSPHATIDYLSERINE FA COMPOSITION OF A SEVO:N-BUTANOL (95:5) DELIPIDATED AND UN-DELIPIDATED HDL FRACTION
DELIPIDATED
UN-DELIPIDATED
nMoles/gram
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
14:0D
15:0
16:0
16:1n7
cis16:1n9
16:0
18:1n9
trans18:2n6
18:3n6
18:4n3
20:1n15
20:1n9
20:2n6
20:4n6
20:3n3
22:0
22:2n6
22:5n6
22:6n3
24:1n9
FATTY ACID

Figure 5

FATTY ACID NOMENCLATURE USED IN FIGURES 1-5